# INTRAGENIC DELETIONS AND SALIVARY BAND RELATIONSHIPS IN DROSOPHILA[1]

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Manuscript received November 8, 1974
Revised copy received December 31, 1974

### ABSTRACT

In the absence of assumptions pertaining to the organization and function of chromomeric DNA, the cytogenetic analysis of intragenic deletions that start at Notch and spread to the right or left of the locus suggests that the recombinational gene is bilaterally associated with salivary band 3C7. Either there are two genes resolved as a single cistron, or one must seek an alternative interpretation that allows some modicum of independent in the relationship between gene and band. Although we momentarily lean toward the hypothesis that gene and salivary band are separate entities on a binemic chromosome, alternative views can be devised, and the data must remain open to reinterpretation.—The recessive visible allele $fa^{swb}$ behaves as a point mutant at the left end of the map and seems to be a deletion in the interval 3C6 to 7; we suspect some part of the band is missing. We have used the aberration in $fa^{swb}$ as a cytological marker, isolated intragenic recombinants, and subjected them to examination. The analysis indicates that the chromosomal interchanges occurred to the right of 3C7.

IN a previous publication, we presented cytogenetic information derived from the analysis of $X$-linked Notch mutants associated with visible deficiencies (WELSHONS 1974). The main thrust centered upon the study of $Df(1)N^{62b1}$ in which a deletion initiated in the Notch cistron and extended to the right some 1.5 map units to the locus of diminutive $(dm)$. Cytologically, $N^{62b1}$ was defined as $Df(1)3C8-3D5$. Recombination studies performed with $N^{62b1}$ and a series of intragenic mutant sites revealed that, whereas $N^{62b1}$ would cross over with the left-most mutant $N^{55e11}$, no recombination would occur with any to the right. $N^{62b1}$ seemed to be deficient for approximately 80% of the right end of the gene, and cytologically, the missing portion of the gene would be confined largely to interband 3C7–8.

Since a deletion at Notch that extends to the left could eliminate the left end of the gene, we investigated two visible deficiencies for the region white $(w)$ through roughest $(rst)$ and verticals $(vt)$ to Notch $(N)$ (see LINDSLEY and GRELL 1968 for descriptions and map positions). One mutant with a deficiency to the left called $N^{68f19}$ did not recombine with the left-most site, but it crossed over with all test mutants to the right. It could be that $N^{68f19}$ lacked the very left

end of the gene. The other left-side deficiency $N^{66i25}$ would not recombine with two widely separated mutants on the left of the genic map, but recombinants were isolated with sites to the right, and $N^{66i25}$ seemed to be deficient for about 80% of the left part of the gene. Because the cytogenetic analysis of the right side $Df(1)N^{62b1}$ indicated that the recombinational gene was interband 3C7–8, it was predicted that the deficiency in the left side $Df(1)N^{66i25}$, starting at $w$ and penetrating deep into the Notch gene, would have to pass through and eliminate 3C7 to reach the interband region 3C7–8. The prediction did not materialize; $Df(1)N^{66i25}$ and $Df(1)N^{68f19}$ both retained 3C7. Cytologically, they were described as $Df(1)3C2–3C6$ and were indistinguishable from $w^{67k30}$ with no lesion at Notch. In the experiments with $Df(1)N^{68f19}$ and $Df(1)N^{66i25}$, some genetic instability was noted that lessened the value of the data; hence, we concluded on the basis of the $Df(1)N^{62b1}$ analysis that a large part of the gene was interband 3C7–8, and the cytology of $Df(1)N^{68f19}$ and $Df(1)N^{66i25}$ indicated that 3C7 was a functional part of the Notch gene.

We have now shown that the instability in the $N^{66i25}$ stock was not associated with the $X$ chromosome, and the genetic data previously obtained were valid. In addition, we have genetic data on a new recessive visible allele at Notch called facet-strawberry ($fa^{swb}$). The new allele is localized at the extreme left-end of the map near $N^{55e11}$. The cytology was surprising because this recessive visible and viable allele seems to be deficient in the interval 3C6 to 7. A consideration of data pertaining to the left side $Df(1)N^{66i25}$, the right side $Df(1)N^{62b1}$ and the $fa^{swb}$ allele suggest that the gene of recombination is not represented as a salivary band.

## MATERIALS AND METHODS

The deficiency chromosomes used for genetic analysis have been described in an earlier publication (WELSHONS 1974), and only a brief reminder is necessary. $Df(1)N^{68f19}$ and $Df(1)N^{66i25}$ are derived from $w^8$ (GREEN 1967). Genetically they are $w^-$, $rst^-$, $vt^-$, and $N$. Cytologically they conform to $Df(1)3C2–3C6$, as does $w^{67k30}$, an X-ray-induced deficiency without a lesion at Notch (LEFEVRE and GREEN 1972).

$Df(1)^{62b1}$ was induced by ionizing radiation and obtained from P. T. IVES. We have described it as $Df(1)3C8–3D5$; it is $dm^-$.

The recessive visible mutant facet-strawberry, symbolized as $fa^{swb}$ (synonym: $swb^{71b}$), was X-ray-induced by LEFEVRE and KELLEY (1972), who demonstrated that it was a recessive visible allele at Notch. The phenotype descriptions that follow were observed at 25°. In males, the mutant resembles a strong allele of facet ($fa$); the eyes are rough and show some variable tendency to be glossy, as in the stronger allele facet-glossy ($fa^g$). The $fa^{swb}$ allele is not dosage-compensated; in homozygous females the phenotype is much reduced, and on occasion, might overlap wild type. In this respect, it resembles the mild mutant allele $fa$. Heterozygotes $fa^{swb}/fa$ and $fa^{swb}/spl$ (split) might have slightly rough eyes, but we cannot be sure; however, $fa^{swb}/fa^g$ females have eye facets that are rough but not glossy. With the wing mutant facet-notchoid ($fa^{no}$), there is often a slight thickening of the longitudinal veins at their junction with the marginal vein. In the genotypes $N^{55e11}/fa^{swb}$ and $N^{264-40}/fa^{swb}$ the eye phenotype is enhanced, the facets are glossy-like, and in addition, the dominant Notch phenotype is more extreme. These heterozygotes with $N$ are relatively inviable and require the addition of $Dp(1;2)51b7$ (LEFEVRE 1952) to the genome to cover the Notch locus and enhance fertility.

From heterozygotes of $fa^{swb}/fa^g$ the cis arrangement was isolated, although we did not expect to detect it. It was assumed that the coupled mutant $fa^{swb} fa^g$ would have an eye phenotype

that looked like $fa^g$ alone, and it does, but a mutant wing phenotype that resembles $fa^{no}$ is expressed in this cis condition; wing venation is thickened as in $fa^{no}$, but unlike $fa^{no}$, the wings are rarely nicked in males. The double mutant wing phene is not dosage-compensated; in females, expression is obviously reduced. Early in our investigation of $fa^{swb}$, D. O. KEPPY discovered, upon examining the salivary gland chromosomes, that the mutant was associated with a chromosomal aberration. Further study has suggested that $fa^{swb}$ is a deficiency in the region 3C6 to 7.

The methods of genetic analysis have been described in detail in the earlier publication. Only in recombination experiments involving an $N$ and $fa^{swb}$ did we significantly alter our standard procedure in response to the inviability of $N/fa^{swb}$ heterozygotes. To overcome the difficulty, we had to cover the mutant function of the Notch locus by incorporating $Dp(1;2)51b7$ into the genome; and in recombination experiments using $N$/recessive visible heterozygotes, the introduction of the autosomally inherited duplication reduces the resolving power of the analysis. Partly to compensate and partly to test another system, we linked the mutants at Notch to $l(1)D43L1$ and $l(1)T2$–$14a$. These two nonallelic lethals are closely linked, positioned at the right end of the $X$ chromosome and remote from Notch. In the following demonstration cross, $l^1 = D43L1$, $l^2 = T2$–$14a$ and the duplication in autosome 2 is represented as $Dp$. Females $\gamma\, w^a + N^{62b1}\, dm^- + l^1/ + w^a\, fa^{swb} + + rb\, l^2$; $Dp/+$ are crossed to $w^a\, fa^g\, fa^{no}\, rb$ males. The cross produces no male offspring since all hemizygotes with or without $Dp$ are lethal (because of the action of $l^1$ or $l^2$). Of the females, half are expected to inherit $Dp$ and survive, and in the remaining half without $Dp$, $N/fa^g\, fa^{no}$ is a lethal condition; only the $fa^{swb}/fa^g\, fa^{no}$ fraction survives and expresses a mutant condition at Notch. Intragenic recombination produces $\gamma\, w^a + + + +$ $rb\, l^2/w^a\, fa^g\, fa^{no}\, rb$ females; $w^a$ and $rb$ interact to produce white eyes, but there is no mutant expression at Notch. The putative recombinant females were progeny tested by mating to a $w^a\, fa^g$ male, and recombination in the females eliminates the loosely linked lethal so that $\gamma\, w^a + + + rb$ males are produced. The lethals $D43L1$ and $T2$–$14a$ were obtained from CHOVNICK's laboratory.

For cytological examination, a lactic-acetic orcein squash technique was used throughout, and all salivary preparations were temporary.

### RESULTS AND DISCUSSION

In the ensuing descriptions we will define a symbolism to describe the cytological conditions of the various deleted chromosomes. $Df(1)N^{66i25}$ is visibly deficient for the region left of Notch, as is $Df(1)N^{68f19}$; both can be characterized as $DfL$-$3C7^+,N$ meaning a deficiency to the left retaining the Notch band; $3C7^+$ has no functional significance. By contrast, $Df(1)N^{62b1}$ can be identified as $DfR$-$3C7^+,N$.

The genetic analysis of $DfL$-$3C7^+,N^{66i25}$ suggested that about 80% of the left end of the Notch gene was missing; but the detection of some instability in the crosses was disconcerting (WELSHONS 1974). The alterations we noted, however, were not directly associated with the $X$ chromosome but with the small duplication of $X$ chromosomal material inserted into autosome 2 and used to cover the lethality of $N^x/N^y$ heterozygotes. In addition to $N^+$, $Dp(1;2)51b7$ carries $w^+$, $rst^+$, and $vt^+$, and the detected changes were to $w^-$ or $(w, rst, vt)^-$. Because a change in $Dp$ was the assay for detection, it could be that the mutability factor from $w^s$ (GREEN 1967) was still with $N^{66i25}$ on the $X$ but capable of transferring its affinity to $Dp$ or the autosomal material closely linked to $Dp$. If so, deficiencies in $Dp$ could arise by the same process that caused $Df(1)N^{66i25}$ to come out of $w^s$. Alternatively, the factor could be wholly associated with autosome 2 and (or) $Dp(1;2)51b7$, in which case we should be able to eliminate it by substitution. We

sent a sample of altered $Dp$'s to LEFEVRE and later received information that, at least in one instance, the duplication and part of the adjacent autosome 2 material was deleted.

Assuming that the mutability factor was segregating with the autosome and not with the $X$, and suspecting that not all individuals in the cross carried it, we isolated one male $Df(1)N^{66i25}$; $Dp/+$, made a new stock from it and began to repeat the experiments starting with $N^{60g11}$ on the right end of the map (Figure 1). At the same time we isolated another male, crossed it so as to produce females heterozygous for the $N$ and a balancer chromosome, then reisolated a male $Df(1)N^{66i25}$; $Dp/+$ with a $Dp$ from an uncontaminated source. The process was repeated a number of times while the initial experiment with $N^{60g11}$ was in progress.

From the first cross, $w^- N^{66i25} + +/w^a + N^{60g11}$ $rb$; $Dp/+$ females by $w^a fa^{no}$ $spl$ males, we isolated 6 $w^a + + +$ chromosomes in 65,800 for a recombination value of .018%. One putative convertant of $N^{60g11}$ was detected. Unfortunately the mutability factor was still present. In one culture bottle, more than 10 alterations were noted, and in two of these cases we know that $w^+$ in $Dp$ mutated to $w^-$ without a change at $rst$ or $vt$. In one other unrelated culture, a single $w^- Dp$ appeared. The multiple isolations from a single culture could represent a single event in one female parent, in which case the germ line could have been almost entirely composed of $w^- Dp$.

In the next cross with $N^{66i25}$, we used the tester site $N^{Co}$ to the left of $N^{60g11}$ (Figure 1) and closer to the presumed deficiency in $N^{66i25}$. By this time it seems we had eliminated the mutability factor and detected 9 recombinants in 55,600 (.032% recombination). In all subsequent crosses no changes in $Dp$ were noted. Heterozygotes of $N^{66i25}$ with $N^{264-40}$ produced 0/86,000 in our preceding report, and in the repeat experiment, the results were again negative: 0/68,600. It seems that the deletion in $DfL$-$3C7^+$, $N^{66i25}$ moves into the Notch gene from the left and terminates to the right of tester site $N^{264-40}$, probably in the vicinity of the recessive allele $spl$ (Figure 1). Negative results are predicted (as previously obtained) from heterozygotes of $N^{66i25}$ with the left-most site $N^{55e11}$. We did not repeat the cross, however, because similar confirmatory data was obtained from heterozy-
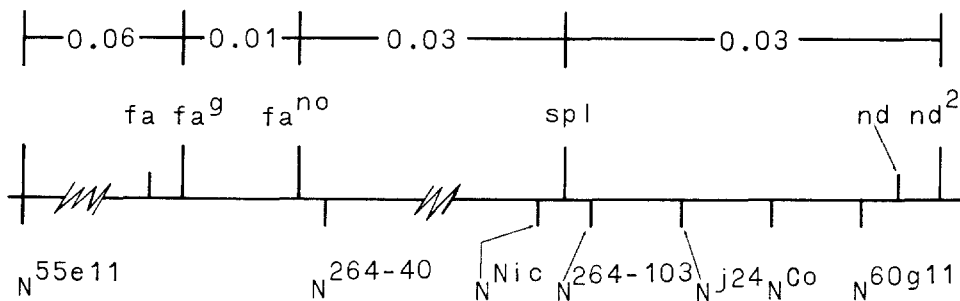


FIGURE 1.—A genetic map of Notch with recessive visible mutant sites represented above the line and recessive lethal $N$'s below.

gotes of $N^{66i25}$ with $N^{62b1}$, but the results are important in a different context and will be described later.

Because the cytology of $Df(1)N^{66i25}$ has remained unchanged and the mutability factor seems to have segregated out of the stock on autosome 2, we conclude that the results previously obtained were valid. Even if the mutability factor is still present in the genome but at a different autosomal site so that we have no assay for detection, it is unlikely that it has affected the recombinational analysis. The cytogenetics of this $DfL$-$3C7^+,N$ suggests that the recombinational gene is positioned in interband 3C6 to 7 or represented as the left edge of 3C7, whereas the $DfR$-$3C7^+,N^{62b1}$ analysis placed at least a significant part of the gene in interband 3C7 to 8. We suspect that the gene can be on either side of the band.

*The genetics of left-side/right-side deficiency heterozygotes*: The published data on $DfL$-$3C7^+,N^{68f19}$ demonstrated that, whereas $N^{68f19}$ recombined with $N^{264-40}$ and additional sites to the right, no recombinants were obtained with the left-most site $N^{55e11}$, suggesting that a deficiency from the left extended into the Notch gene up to and possibly beyond $N^{55e11}$. From the genetic map we estimate a bit more than .07 map units between $N^{55e11}$ and $N^{264-40}$ (Figure 1). The recombination value obtained from $N^{68f19}/N^{264-40}$ was .098, and allowing for inaccuracies in the estimates, the data indicate that the deletion in $N^{68f19}$ did not extend into Notch much beyond $N^{55e11}$ (WELSHONS 1974). The right-side deficiency $N^{62b1}$ analysis indicated that this deletion retained only a small segment of the gene (estimated as .023 map units) in the interval $N^{55e11}$ to *fa* (Figure 1); hence, recombination might be detected in heterozygotes $DfL$-$3C7^+,N^{68f19}/DfR$-$3C7^+$, $N^{62b1}$ since the trans condition of the deficiencies seemed to retain a small intact segment at the left end of the gene.

From heterozygotes $\gamma\ w^-\ N^{68f19}\ +\ +/\gamma\ w^a\ +\ N^{62b1}\ dm^-;\ Dp/+$ females by $w^a$ $fa^{no}\ spl$ males, R. J. WELTER in this laboratory first isolated two $\gamma\ w^a\ +\ +\ +$ recombinant chromosomes in 65,100 (recombination value = .006%). Later, we repeated the experiment and obtained 4/77,600, for a value of .010%; one $w^-$-type alteration in $Dp$ was detected, but we do not believe the possible presence of an autosomally transmitted mutability factor in the $Df(1)N^{68f19}$ stock (out of $w^s$) jeopardizes the conclusion that this $DfL$-$3C7^+,N/DfR$-$3C7^+,N$ heterozygote is recombinationally competent. The deficiencies are non-overlapping in part of the interval $N^{55e11}$ to *fa*.

Cytological analyses of male salivary glands have shown that both $N^{68f19}$ and $N^{66i25} = DfL$-$3C7^+,N = Df(1)3C2$-$3C6$ (WELSHONS 1974). Both retain 3C7 (in whole or in part), but differ genetically. The left-side deficiency in $N^{68f19}$ terminates around $N^{55e11}$, whereas in $N^{66i25}$ the deletion probably ends farther to the right, beyond $N^{264-40}$. From the previous type of cross, $DfL$-$3C7^+,N/DfR$-$3C7^+$, $N$, recombinants were predicted on cytological and genetical bases in $N^{68f19}/N^{62b1}$ heterozygotes, since both were 3C7$^+$ and the deletions were genetically non-overlapping. In the same type of cross, the $N^{66i25}/N^{62b1}$ heterozygous deletions overlap genetically but retain 3C7. Our data suggest that the $DfL$-$3C7^+,N^{66i25}/DfR$-$3C7^+,N^{62b1}$ condition should be heterozygously deficient at opposite ends of the Notch gene and homozygously deficient for an interstitial piece; hence, we

would not expect to isolate recombinants. The cross $+ w^- N^{66i25} +/\gamma w^a N^{62b1}$ $dm^-$; $Dp/+$ by $w^a fa^{no} spl$ was negative, 0/91,000.

From the analysis of data in our previous publication, we suggested that exchanges between salivary bands (chromomeres) might make only a limited contribution to genetic length. In this one case, the results are supportive. One can argue that the trans condition for the heterozygous deficiencies has simply inhibited crossing over; but at the moment, we can only point to the isolation of recombinants from the same type of left-side, right-side cross, $DfL$-$3C7^+$,$N^{68f19}$/ $DfR$-$3C7^+$,$N^{62b1}$.

The left-side deficiencies $N^{68f19}$, $N^{66i25}$, and three others presently under investigation, place the recombinational gene at the left edge of 3C7 or interband 3C6 to 7. Only $DfR$-$3C7^+$,$N^{62b1}$ suggests that the gene is to the right of the band, and an error in the cytology could have the effect of placing $N^{62b1}$ to left side with the other Notches so far analyzed. If we assumed that the breakpoints yielding $Df(1)N^{62b1}$ occurred on the left between 3C6 and 7 and the one on the right evenly split the 3D5,6 band, then $N^{62b1}$ could be defined as $Df(1)3C7$-$3D5$. The band identified as 3C7 in $N^{62b1}$ would represent 3D6, but an adjacent fine band, thought to represent the very right edge of 3D6 in our published interpretation, would either be an artifact or a band between 3D5,6 and 3E1,2 that did not appear on BRIDGES' map. Hoping to obtain additional cytological support for our original interpretation, we synthesized the cis arrangement $Df(1)w^{67k30} Df(1)$-$N^{62b1}$. From the cross $+ w^{67k30} + +/\gamma w^a N^{62b1} dm^-$ by $w^a spl$, we isolated three double deficiencies (and three of the reciprocal $\gamma w^a$ recombinants). $Df(1)w^{67k30}$ $= Df(1)3C2$-$3C6$ and on the basis of our published definition of $Df(1)N^{62b1} =$ $Df(1)3C8$-$3D5$ (and most of 6), the double deficiency should have three clearly discernible bands: 3C1, 3C7, and 3E1,2. All three linked deficiencies looked the same (Figure 2). There should also be a fine band to right of 3C7, but since it is difficult to visualize, we will not attempt to deal with it in the cytological analysis. If the band in $N^{62b1}$, thought to be 3C7, is really 3D6, the order of bands then becomes 3C1, 3D6, and 3E1,2.

We examined salivary gland preparation of female heterozygotes $w^{67k30}/$ $w^{67k30} N^{62b1}$. The pairing configurations are distorted because $w^{67k30}$ carries material to the right of 3C7 that is not represented in the other homolog, but we hoped to obtain some clear views at the left and right ends of the distorted segment. We saw several cases where 3C1 and 3C7 in $w^{67k30}$ were clearly and neatly paired, separable from each other and from 3C9,10 in $w^{67k30}$ which had no pairing partner. We saw a few cases where the band presumed to be 3C7 in the double deficiency moved off in the direction of 3D6 but did not seem to reach it. These could represent cases where pairing in the intact region 3D6 to 3E1,2 was facilitated by lack of pairing at 3C7. The observations add only a bit of support for our original interpretation of $N^{62b1}$ because it is easy to generate other explanations for the observations. It could be that bands of different origin will pair nonhomologyously in abnormal situations; or two nonhomologous bands lying opposite each other might remain unpaired in the living condition and simply conjoin upon fixation. We will continue with the view that $N^{62b1}$ retains 3C7, and
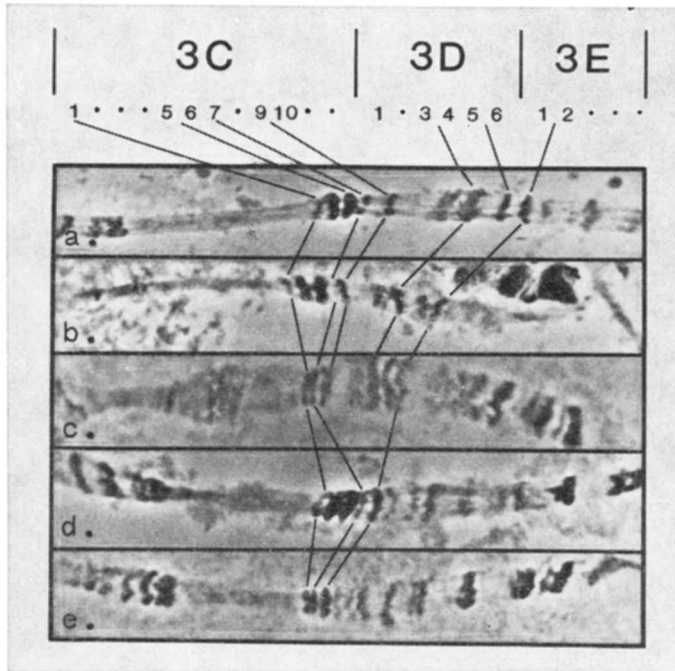
FIGURE 2.—Salivary gland preparations of normal and deficiency chromosomes obtained from male larvae. (a) The $X$ of wild type. (b) The recessive $fa^{swb}$ demonstrating the absence of a typical band at the 3C7 position. (c) $Df(1)w^{67k30}$; $Dp/+$ missing bands 3C2 to 3C6. (d) $Df(1)N^{62b1}$; $Dp/+$ demonstrating the deficiency between bands 3C7 and 3E1,2. (e) The coupled deficiencies in $w^{67k30}$ $N^{62b1}$; $Dp/+$ males.

we take considerable comfort in the fact that a number of $N$'s are associated with breaks to the right of 3C7 (LINDSLEY and GRELL 1968; BEERMAN 1972), and that the inversion in $N^{66h26}$ (WELSHONS 1974) seems to have one of its breakpoints to the right of 3C7.

*The genetics of* fa[swb]: This recessive mutant is associated with a cytological aberration interpretable as a deficiency in the 3C6 to 7 region. The genetic crosses describe its relationship to the region of the genic map left of $fa^g$ (Figure 1). Heterozygotes $w^a + fa^g +/+ fa^{swb} + rb$ by $w^a + fa^g rb$ males produced 26 $w^a + +$ $rb$ recombinants/78,700 tested chromosomes, a recombination value of .066%. The double mutant recombinant $+ fa^{swb} fa^g +$ was also detected in the male progeny owing to the unexpected appearance of a mutant wing phenotype (see MATERIALS AND METHODS). Since $fa^{swb}$ was associated with a cytological aberration, and suspecting that $fa^{swb}$ might be separable from it, we examined 3 $+ fa^{swb}$ $fa^g +$ and 2 $w^a + + rb$ intragenic recombinants. The double mutant had $fa^{swb}$ cytology and the wild types were normal. Two $w^a fa^{swb} + rb$ from an exchange to the left of $fa^{swb}$ were also examined, and they too had $fa^{swb}$ cytology. To make certain that we really had isolated the double mutant expressing a wing abnormality in the cis condition, we crossed $w^a + + +/+ fa^{swb} fa^g rb$ to wild-type males and screened the male progeny. We isolated 12 $+ fa^{swb} + +$ and 9 $w^a +$

$fa^g$ $rb$ males in a sample of 19,000. The recombination value $= .111\%$. The allele $fa^{swb}$ was to the left of $fa^g$ in the vicinity of $N^{55e11}$.

Representing two tightly linked recessive lethals at the right end of the $X$ chromosome (remote from Notch) as $l^1$ and $l^2$ (see MATERIALS AND METHODS), we crossed $y\ w^a\ N^{55e11} + l^1/+ w^a\ fa^{swb}\ rb\ 1^2, Dp/+$ females to $w^a\ fa^g\ fa^{no}\ rb$ males. No recombinants were detected in 64,500 tested chromosomes, suggesting that $fa^{swb}$ is tightly linked to, or inseparable from, $N^{55e11}$. If so, we might detect recombination with $fa^{swb}$ and $DfR\text{-}3C7^+, N^{62b1}$ since $N^{62b1}$ retains a small piece of the map in the $N^{55e11}$ to $fa$ interval. From heterozygotes $y\ w^a + N^{62b1}\ dm^- +$ $l^1/+ w^a\ fa^{swb} + + rb\ l^2;\ Dp/+$, we obtained 3 $y\ w^a + + + rb$ chromosomes in 52,500 (recombination value $= .011\%$). Two recombinants were examined cytologically and both were normal.

With $fa^{swb}$ in the neighborhood of $N^{55e11}$, we wished to test it against $Df(1)w^{67k30} = Df(1)3C2\text{-}3C6$. Since $fa^{swb}$ seemed to be deficient in the Notch region, we wanted to isolate the coupled mutants $w^{67k30}\ fa^{swb}$ because this recombinant (double deficiency) could have some cytological pertinence. Representing $Df(1)w^{67k30}$ as $w^-$, we performed the cross $w^- + +/+ fa^{swb}\ fa^g$ by $w^a\ fa^{swb}\ fa^g$. The reciprocal recombinant chromosomes, $+ + +$ and $w^-\ fa^{swb}\ fa^g$, would come about by exchange in the region $w^-$ to $fa^{swb}$. The wild type would survive in male or female, but the $w^-\ fa^{swb}\ fa^g$ would survive only in the female. We would also detect recombinants in the intragenic interval $fa^{swb}$ to $fa^g$. No crossovers were detected in the region to the left of $fa^{swb}$, but we did obtain 18 $+ fa^{swb} +$ hemizygous males and 9 $w^- + fa^g$ chromosomes (recoverable only in females). We examined about 143,000 progeny, and allowing for the fact that $w^-$ does not survive as a male, screened 191,000 chromosomes. The recombination value $fa^{swb}$ to $fa^g$ was $.028\%$, low compared with our previous data. The 9 $w^- + fa^g$ chromosomes were $Df(1)3C2\text{-}3C6$ as expected, and 3 $+ fa^{swb} +$ recombinants were cytologically aberrant like all $fa^{swb}$'s examined to date. The experiment produced some oddities. Five $w$ males were detected and cytological examination clearly indicated that they were intrachromosomally duplicated, most likely originating from nonhomologous pairing and exchange. One $w\ fa^{swb}\ fa^g$ male, representing a spontaneous mutation at the white locus, had the typical $fa^{swb}$ cytology.

We used the $w^- + fa^g$ chromosome in another attempt to isolate crossovers to the left of $fa^{swb}$. Heterozygotes $w^- + fa^g/+ fa^{swb} +$ were crossed to $w^a + fa^g$ males, and we did recover 2 $+ + fa^g$ chromosomes, with normal cytology, resulting from exchange in the region $w^-$ to $fa^{swb}$, but the coupled condition $w^-\ fa^{swb}$ was not detected. The progeny numbered 82,300, equal to approximately 109,700 tested chromosomes. The recombination value $w^-$ to $fa^{swb} = .002\%$, that for $fa^{swb}$ to $fa^g = .08\%$.

To summarize, $fa^{swb}$ is to the left of $fa^g$ and close to $N^{55e11}$. It recombines with the right-side deficiency $N^{62b1}$ and is separable from the deficiency to the left in $w^{67k30}$, but the linkage is very tight. In Figure 3, we have represented the six chromosomes used in our deficiency analysis: $N^{55e11}$ is shown as a small, barely interstitial deletion at the left end of the map, although we have no direct information that it is; but when it is viewed this way, one can consult the figure,
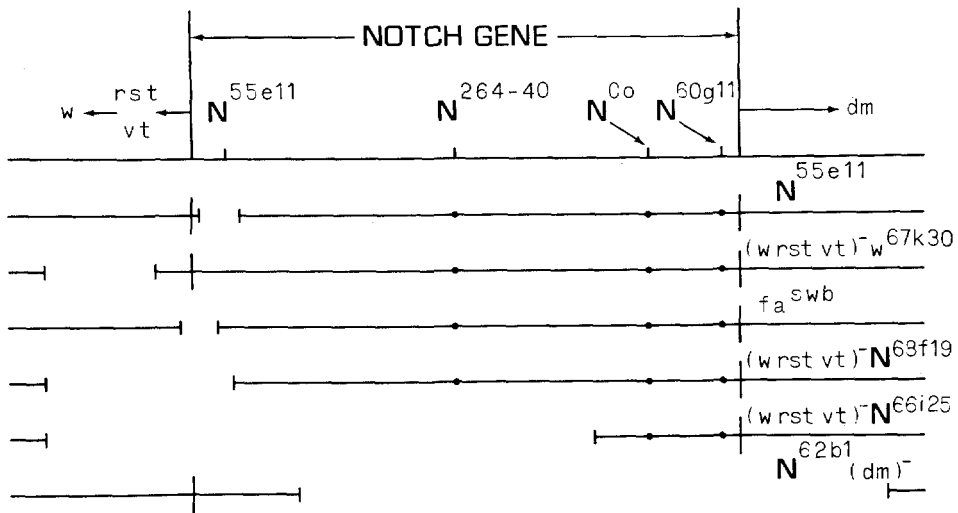
NOTCH GENE

$w \leftarrow \dfrac{rst}{vt}$    $N^{55e11}$    $N^{264\text{-}40}$   Co   $N^{60g11}$    $\rightarrow dm$

$N^{55e11}$

$(w\,rst\,vt)^{-}w^{67k30}$

$fa^{swb}$

$(w\,rst\,vt)^{-}N^{63f19}$

$(w\,rst\,vt)^{-}N^{66i25}$

$N^{62b1}\,(dm)^{-}$

FIGURE 3.—The relationship of genetic deletions to the Notch gene. $N^{55e11}$ is shown as an interstitial deletion, but it may be a point mutant that has not recombined with $fa^{swb}$ or $N^{63f19}$. See text for the equivocal representation of $fa^{swb}$ as a deletion.

mentally construct heterozygotes of any two of the six chromosomes, and discern those crosses that have either yielded or failed to yield recombinants in 10 of the possible 15 heterozygotes tested in this and the preceding report (WELSHONS 1974). In five combinations, $w^{67k30}/N^{63f19}$, $w^{67k30}/N^{66i25}$, $fa^{swb}/N^{63f19}$, $fa^{swb}/N^{66i25}$ and $N^{63f19}/N^{66i25}$, negative results are predicted.

The *cytology of* $fa^{swb}$: As presented in Figure 2, the chromosome seems to be deficient for salivary band 3C7. We have examined many preparations with different degrees of stretch. Often, 3C5,6 seems to be a little too thick; on other occasions something seems to protrude from it. Rarely, we have seen a faint band between 3C5,6 and 3C9,10; it might represent a remnant of 3C7 or it might be 3C8. One thing is certain, there is no readily visualized band in the position where 3C7 ought to be. The cytology can be interpreted in a number of ways, but we will consider only three and identify one we prefer.

It could be that the aberration represents a single band deletion for 3C7, and the breakpoint to the left of 7 has somehow altered the appearance of the heavy band 3C5,6 so that it looks thick and protuberant. If 3C7 has been neatly excised, we can account for the $+ + fa^g$ recombinants with normal cytology that were obtained from $w^- + fa^g/+ fa^{swb} +$ heterozygotes. It seems they were derived by crossing over in the short region between $w^-$ and $fa^{swb}$. Since $w^- = Df(1)3C2\text{-}3C6$, one can presume that some piece of interband 3C6 to 7 is retained in the $w^-$ chromosome and available for exchange with an homologous interband region remaining in $fa^{swb}$. Although recombinant chromosomes carry $fa^g$, they would be cytologically normal. This interpretation of $fa^{swb}$ as a single band deletion suggests, however, that the Notch band lacks a vital function, and since

a functional deletion at Notch is a recessive lethal, it implies that the gene is not in the band.

With reference to the cytological preparation of a normal chromosome pictured in Figure 2, one can imagine a left breakpoint that splits 3C5,6 and another at the left margin of 3C7, in which case the band thought to be 3C5,6 would actually represent a fusion of 5 with 7. From the analysis of LEFEVRE and GREEN (1972), one would expect the deletion for 3C6 to express a verticals ($vt$) phenotype in heterozygotes with $w^{67k30}$ ($w^-$), but it does not. Although some interband immediately adjacent to 3C7 in $fa^{swb}$ could be available for exchange with $w^-$, it seems that the $+ + fa^g$ recombinants would have a $fa^{swb}$-like cytology.

In the last interpretation to be presented, the cytology of $fa^{swb}$ can be generated by one breakpoint to the right of 3C6 and another at the left edge of 3C7; the deletion could be entirely interband or there could be some loss of 7. The band 3C5,6 could appear to be fused with some or all of 7—this might explain the abnormalties seen in the heavy band. Since 3C5,6 would be intact, there could be some interband adjacent to 6 available for exchange with $w^-$, and the recombinant chromosomes would have a normal cytology. We favor this last definition of the deletion.

As implied by the foregoing discussion and description of the $fa^{swb}$ genetics, we have used the chromosomal aberration as a cytological marker in recombination experiments. By isolating intragenic exchanges and subjecting them to cytological analysis, it seems that one should be able to identify the region of chromosomal interchange. For example, $w^a + fa^g + / + fa^{swb} + rb$ heterozygotes yielded $w^a + + rb$ and $+ fa^{swb} fa^g +$ chromosomes by exchange between the alleles $fa^{swb}$ and $fa^g$. Because our genetic analysis implied no exchange within bands, it seems that interallelic crossing over would have to occur in the interband regions to the left or right of 3C7. Exchanges to the right of 7 would yield $w^a + + rb$ chromosomes with normal cytology and $+ fa^{swb} fa^g +$ recombinants with $fa^{swb}$ cytology (as observed). If the $fa^{swb}$ deletion has one breakpoint to the right of 3C6 and the other in the left part of 7, the only interband available for exchange on the left side of the band would be adjacent to 6, but the cytological expectations would be reversed.

One can presume that the deletion in $fa^{swb}$ is completely interband 3C6 to 7. Then it is possible to generate recombinants with the observed cytology by exchange in the left interband immediately adjacent to 7, or in the undisturbed interband to the right. Because $fa^{swb}$ is a lesion in the Notch gene, it is tempting to conclude that the band is directly involved; alternatively, $fa^{swb}$ might be a point mutant closely linked to a deficiency.

From heterozygotes $w^{67k30}/fa^{swb}$, we had hoped to isolate the double mutant that would come about by an exchange in the short region between the two deficiencies. Since $Df(1)w^{67k30}$ retains only 3C1 and 3C7, the cis arrangement might retain only 3C1 if 7 is completely deleted in $fa^{swb}$, or 3C1 plus a remnant if 7 is partly deleted, or 3C1 and 7 if $fa^{swb}$ retains virtually all of 3C7. Crossing over did occur, but we isolated only the wild-type reciprocal event. If we can devise a more sensitive cross, we will try again.

CONCLUSIONS

Attempts to relate the recombinational gene and band suffer from the fact that cytogenetic inferences are drawn from aberrant cytology when the organization and function of chromomeric DNA is conjectural, and the definition of chromosomal deletions is quantitatively imprecise or clearly equivocal. An interpretation of the data will be presented, but to emphasize its tentative nature, we will conclude by identifying critical points where cytological errors or assumptions pertaining to the organization and function of chromomeres could drastically alter the situation.

*Interpretation:* The genetic analysis of the two deficiencies identified as $DfL$-$3C7^+,N$'s places the recombinational gene in interband 3C6 to 7 or the left edge of 3C7, whereas the $DfR$-$3C7^+,N$ data (WELSHONS 1974) placed the gene mostly in the interband 3C7-8, although there could be some involvement at the right edge of the band. The two left-side deficiencies ($N^{68f19}$ and $N^{66i25}$) heterozygous with the right-side deletion ($N^{62b1}$) were both cytologically nonoverlapping, but only the genetically nonoverlapping condition ($N^{68f19}/N^{62b1}$) yielded recombinants. We interpret the data to mean that the gene is not in the band. From the bilateral association of Notch with 3C7, one could conclude that there are two Notch genes; but it is difficult to imagine how they become resolved as one genic map.

We feel that cytological observations on polytene (DERKSEN and SORSA 1972; SORSA 1972, 1973a, 1973b) and mitotic (SORSA 1973c) chromosomes of *Drosophila melanogaster* suggest a useful hypothesis relevant to genes and bands. In the electron micrographs there are two aligned longitudinal fibrils, but only one is associated with a chromomeric loop. We suggest that the recombinational gene is contained on the fibril opposite the chromomere. This view is not novel because HENDERSON (1971a, 1971b) has already proposed a chromosome model for mitotic and meiotic chromosomes incorporating this double-fibril feature. He suggests that the master gene (our recombinational gene) lies opposite the chromomeric strand containing the slave units; but since it requires additional postulates to account for sizable genetic deletions associated with visibly undiminished salivary bands, we remain silent on matters pertaining to the composition of the chromomere. Given such a chromosome model, one can imagine that our left- and right-side deficiencies arose by diagonal and uneven breakage in the two aligned fibrils, deleting the left or right portions of the gene but leaving the band visibly undiminished. If the chromomere does not maintain a fixed position relative to the gene, the breaks need not be diagonal. Allowing for uncertainty in the cytological representation of $fa^{swb}$, this mutant might represent a small deletion or point mutant in the gene associated with a partial deficiency for the chromomere. Alternatively, the mutant phenotype might be due to an interaction between mutant site and chromomeric deletion, implying a functional attribute of the band.

If the postulated band-gene relationship in the polytene chromosome truly represents a highly magnified view of the genetic material in the germ line, then

meiotic chromosomes would not have a unineme structure. Nevertheless, we feel the hypothesis has merit because it readily predicts some results that might be forthcoming in our continuing investigation. We have used left- and right-side deficiencies symbolized as $Df L\text{-}3C7^+$,$N$ and $DfR\text{-}3C7^+$,$N$ in our analysis. It should also be possible to produce and analyze $DfL3C7^-$,$N$'s and $DfR\text{-}3C7^-$,$N$'s with no 3C7 band. For example, an $N$ mutant cytologically described as $Df(1)\text{-}3C2\text{-}3C7$ would represent $DfL\text{-}3C7^-$,$N$. In the event the gene is not completely gone, we should be able to detect an intragenic deletion left and retention right; some $DfR\text{-}3C7^-$,$N$'s should be characterized as deficiency right, retention left. Induced single-band deficiencies for 3C7 could have deletions left, right, or wholly interstitial, and some $N$ mutants should be cytologically normal and yet be genetically resolved as left, right, or interstitial deletions.

The data by SORSA, GREEN and BEERMANN (1973) placed $w$ in interband 3C1 to 2 or the left edge of 2, whereas $sa$ (sparse arista, RAYLE and GREEN 1968) was interband to the left of 3C1 or associated with a minute piece of 3C1. Unlike Notch, there seemed to be a unilateral relationship to the respective bands. By examination of data compiled in LINDSLEY and GRELL (1968), we hoped to find some indication of bilaterality at $w$, but we failed. There are 13 examples of deficiencies extending to the right through Notch with a left break between 3C2,3 and 3C5,6, but none of them involved $w$. It could be that heavy bands like 3C2,3, recorded as doublets by BRIDGES, are associated with complex loci (WELSHONS 1965), and the expectations derived from Notch are not applicable in such cases. There is genetic complexity in the region, as suggested by the roughest ($rst$)-verticals ($vt$) analysis (LEFEVRE and GREEN 1972), which demonstrated interaction between bands 3C3 and 3C5,6. We would expect a bilateral relationship at $sa$, but the data are insufficient for comparison.

*Equivocations:* We feel that the genetics of Notch deletions is the most unequivocal feature of our data; even so, the identification of intragenic deficiencies rests upon the failure to obtain recombinants from appropriate heterozygotes, and one would certainly feel more secure if conclusions were not based on negative results. Granting genetic validity, the cytogenetic analysis of $DfL\text{-}3C7^+$, $N^{66i25}$ and $DfR\text{-}3C7^+$,$N^{62b1}$ suggests that the intragenic deletions in these two $N$'s are localized to the left and right interbands and(or) respective interfaces between 3C7 and interbands. Each is deficient for a considerable piece of the recombinational gene; and in heterozygotes, the deletions overlap, but we cannot detect a diminution of 3C7. Hence, there seems to be a bilaterality in the gene-band relationship. At this point substantial equivocation arises.

We have assumed that visibility undiminished salivary bands are lacking little if any DNA. We could be wrong. We have made no assumptions relating to the organization and function of chromomeric DNA but it is possible to devise a scheme whereby the bilaterality at Notch disappears, and the gene can be placed in 3C7 ;at this time, we are reluctant to make the assumptions. There is also the problem associated with the right-side deficiency $N^{62b1}$; an error in the cytological definition could place it to the left of 3C7 and eliminate the postulate of bilaterality. Obviously, our data must remain amenable to reinterpretation.

If there is a bilaterality at Notch and the locus is composed of one cistron, then gene and chromomere might represent separate entities on a binemic chromosome. This view implies greater complexity in chromosome structure than we like to accept, but we do not feel that it should be ignored. Tentative acceptance of the condition does provide an hypothesis suggesting one experimental approach. If the data fail to support the binemic concept, it is likely that they will enable us to eliminate equivocal interpretations of our cytogenetic analysis.

## LITERATURE CITED

BEERMANN, W., 1972  Chromomeres and genes. In: *Developmental studies on giant chromosomes*. Results and Problems in Cell Differentiation, Volume 4. pp. 1–33. Edited by W. BEERMANN. Springer-Verlag, Berlin.

DERKSEN, J. and V. SORSA, 1972  Whole mount electron microscopy of salivary gland chromosomes after treatment with alkali-urea. Exptl. Cell Res. **70**: 246–248.

GREEN, M. M., 1967  The genetics of a mutable gene at the white locus of *Drosophila melanogaster*. Genetics **56**: 467–482.

HENDERSON, S. A., 1971a  Grades of chromatid organization in mitotic and meiotic chromosomes. I. The morphological features. Chromosoma **35**: 28–40. ——, 1971b  Grades of chromatid organization in mitotic and meiotic chromosomes. II. Their interpretation in terms of a master/slave model. Chromosoma **35**: 41–56.

LEFEVRE, G., JR., 1952  $Dp(1;2)w^{51b7}$. Drosophila Inform. Serv. **26**: 66.

LEFEVRE, G., JR. and M. M. GREEN, 1972  Genetic duplication in the *white-split* interval of the X chromosome in *Drosophila melanogaster*. Chromosoma **36**: 391–412.

LEFEVRE, G., JR. and J. KELLEY, 1972  Strawberry *vs.* facet-glossy, a locus correction. Drosophila Inform. Serv. **48**: 146–147.

LINDSLEY, D. L. and E. H. GRELL, 1968  Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **627**.

RAYLE, R. E. and M. M. GREEN, 1968  A contribution to the genetic fine structure of the region adjacent to white in *Drosophila melanogaster*. Genetica **39**: 497–507.

SORSA, V., 1972  Whole mount electron microscopy of core fibrils in salivary-gland chromosomes of *Drosophila melanogaster*. Hereditas **72**: 169–172. ——, 1973a  Whole mount electron microscopy of small chromomeres in salivary gland chromosomes of *Drosophila melanogaster*. Hereditas **73**: 143–146. ——, 1973b  Whole mount electron microscopy of fibril rings in alkali-urea treated salivary gland chromosomes of *Drosophila melanogaster*. Hereditas **73**: 147–151. ——, 1973c  Core fibrils in early mitotic prophase chromosomes of *Drosophila melanogaster*. Hereditas **74**: 147–151.

SORSA, V., M. M. GREEN and W. BEERMANN, 1973  Cytogenetic fine structure and the chromosomal localization of the white gene in *Drosophila melanogaster*. Nature New Biol. **245**: 34–37.

WELSHONS, W. J., 1965  Analysis of a gene in Drosophila. Science **150**: 1122–1129. ——, 1974  The cytogenetic analysis of a fractured gene in Drosophila. Genetics **76**: 775–794.